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Altered gut microbiota and mucosal immunity in patients with schizophrenia

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ABSTRACT

Evidence shows that gut microbiota may play important roles in schizophrenia pathogenesis via the "gut-brain" axis, but the mechanisms remain unclear. Here, eighty-four patients with schizophrenia and 84 sex- and age-matched healthy controls were enrolled. Shotgun metagenomic sequencing and 16S rRNA sequencing were performed, and the gut microbiota-associated epitopes (MEs) were predicted, which, together with IgA content, were used to determine the gut microbiota composition associated with gut immune status. Patients with schizophrenia had significantly reduced gut microbiota richnesses compared with those of the healthy controls, and the gut microbiota compositions clearly distinguished the patients with schizophrenia from the healthy controls. Based on two-stage metagenomic-wide association studies, nineteen gut microbiota taxonomies were associated with schizophrenia, and the microbial dysbiosis (MD) index was calculated based on the abundance of differential taxonomies. We found that MD index was positively correlated with MEs diversity and gut IgA levels, and negatively correlated with gut microbiota richness. Glutamate synthase (GOGAT) was more active in the guts of patients with schizophrenia than in those of healthy controls, and high GOGAT activity was associated with altered gut microbiota taxonomies associated with gut IgA levels. Our results may imply a role of the microbiome in the etiology of schizophrenia and contribute to the development of microbiome targeted interventions for schizophrenia.

1. Introduction

Schizophrenia, a serious mental disorder affects how a person thinks, feels and behaves, affects approximately 1% of the population (Saha et al., 2005). With the development of chlorpromazine in the 1950s, antipsychotic treatments began to appear and are currently used today. However, treating schizophrenia is challenging because it is treatment-resistant (Nucifora et al., 2018). The gut microbiota may play an important role in the schizophrenia pathogenesis via the "gut-brain" axis, and understanding its specific role will provide new approaches to treating schizophrenia (Dinan and Cryan, 2018; Nguyen et al., 2018). Prenatal infection is an important risk factor for schizophrenia (Brown and Derkits, 2010), and studies have shown that offspring who experience maternal immune activation (MIA) during pregnancy exhibit schizophrenia-like behavior (Van den Eynde et al., 2014; Wolff et al., 2011). Increasing evidence shows that the gut microbiota may be involved in the development of schizophrenia through immune and inflammatory mechanisms (Dickerson et al., 2017). The functional composition of the gut microbiota should be systematically studied in patients with schizophrenia, especially the composition of the immune-

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associated gut microbiota. It is well known that antigenic proteins can be processed by the body to form a series of peptides, also known as immune epitopes, which are key components of the body's ability to recognize antigens and induce adaptive immune responses (Sakaguchi, 2004); it is speculated that gut microbiota-associated epitopes (MEs) may mediate the body's immune response to the gut microbiota. We previously developed a method to predict the gut immune composition based on shotgun metagenomic sequencing data and used gut IgA levels as indicators of gut microbiota-associated epitopes (MEs) (Wang et al., 2019). In the present study, we enrolled eighty-four patients with schizophrenia and 84 sex- and age-matched healthy controls and subjected them to shotgun metagenomic sequencing and 16S rRNA sequencing in two phases. Based on metagenome-wide association analysis, we identified the schizophrenia-associated gut microbiota composition and predicted the MEs and IgA content in the gut. The intestinal flora's immune function was measured, and the intestinal IgA content was used to determine whether the gut microbiota plays role in schizophrenia pathogenesis via immune mechanisms.

2. Materials and methods

2.1. Participants

Participants were recruited from Longgang Central Hospital of Shenzhen and Shenzhen Kangning Hospital in Shenzhen, China. Patients with schizophrenia were included in the study if they had been diagnosed with schizophrenia as per the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (Arlington, 2013). Patients and controls were excluded if they had genetic metabolic diseases, brain injury, acute physical illness, antibiotic use, and/or drug use for gastrointestinal diseases in the 2 weeks prior to starting the study. Healthy controls were included who were age- and sex-matched and unrelated to the patients with schizophrenia. A questionnaire was used to survey the diet and lifestyle habits according to our previous study (Wang et al., 2019), the questionnaire contains >50 questions about BMI, diet and lifestyle, and mental health issues. This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Longgang Central Hospital of Shenzhen (No. 2016016).

2.2. Sample collection

The researchers distributed the disposable sterile potty and tubes to the participants in advance, participants first discharged the feces in a sterile potty, then washed their hands, put on disposable gloves, and took the middle part of the feces. Feces samples were immediately stored in a −20 °C freezer. On the same day or the next day, the researchers collected the sample and stored it in a −80 °C freezer. The StoolGen fecal DNA extraction kit (CWBiotech, Beijing, China) was used to extract the genomic DNA.

2.3. Shotgun metagenome sequencing

Shotgun metagenome sequencing was performed as described in our previous studies (Zhou et al., 2019; Zhou et al., 2016) and detailed in the Supplementary Appendix. Briefly, reads with poor quality or containing human sequences were filtered out, and MEGAN5 (Huson et al., 2007) was used for metagenomic profiling at the taxonomic and functional levels.

2.4. Gut microbiota-associated epitope (ME) analysis

Gut MEs were predicted as previously described (Wang et al., 2019). Briefly, the Immune Epitope Database (IEDB), version 3.0 (Vita et al., 2015), was downloaded, and a reference database was constructed using the formatdb function in BLAST (Altschul et al., 1990). The shotgun metagenome reads without human sequences were blasted against the reference database using the BLASTX function in BLAST with the default parameters, or evalue 0.01 was used as cut-off, and the results with best hits, which rank first in the BLASTX output, usually the best bitscore or e-value were retained and counted as ME datasets.

2.5. 16S rRNA gene sequencing and analysis

16S rRNA gene sequencing was performed as described elsewhere (Zhou et al., 2018b) and detailed in the Supplementary Appendix. Briefly, the V4 region of the bacterial 16S rRNA gene was amplified with 515F and 806R primers by polymerase chain reaction; the libraries were constructed using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) and sequenced using HiSeq2500 (Illumina). The raw reads were merged and filtered into clean reads using Quantitative Insights Into Microbial Ecology (QIIME, version 1.9.1) (Caporaso et al., 2010). Operational taxonomic units (OTUs) with ≥97% similarity were determined using Uparse (version 7.0.1001) (Edgar, 2013) and annotated using the SILVA database (Quast et al., 2013). The OTUs were normalized using USEARCH (v10.0.240) "-otutab_norm" command based on the least data size of samples before analysis, then we do OTU analysis and normalize OTU data to 100% for each sample.

2.6. Diversity analysis

Gut microbiota diversity and ME alpha diversity were analyzed using the vegan package diversity function. Chao 1 diversity was computed using the vegan package estimateR function; the diversity index was demonstrated using the boxplot function in the R package. The Wilcoxon rank-sum test in R's wilcox.test function was used to determine the difference in diversity indexes between groups, and significance was defined as: *$p < 0.05$, **$p < 0.01$, or ***$p < 0.001$. To analyze the gut microbiota diversity and ME beta diversity, a non-metric multidimensional scaling (NMDS) analysis was performed using the metaMDS function in the vegan package.

2.7. Metagenome-wide association study

A metagenome-wide association study (MWAS) was performed as previously described (Qin et al., 2012; Wang et al., 2019). The criteria for significant differences in gut microbial compositions at the taxonomic level were as follows: average relative abundance >0.01%, coverage >0.5, false discovery rate (FDR) corrected p value ≤0.05 for both the Wilcoxon rank-sum test and the Deseq2 test, and absolute value of log2 (fold change) ≥0.2. The criteria for significant differences in gut microbial composition at the functional level was as follows: average relative abundance >0.05%, coverage >0.5, FDR corrected p value ≤0.05 for both the Wilcoxon rank-sum test and Deseq2 test, and absolute value of log2 (fold change) ≥0.2. The criteria for significant differences in MEs was as follows: average relative abundance >0.03%, coverage >0.5, FDR corrected p value ≤0.05 for Fisher's exact test.

2.8. Correlation and regression analyses

To determine the correlation between gut microbiota taxonomies and functional pathways or MEs, the Spearman rank correlation coefficient was calculated using the cor and cor.test functions in R. The correlation coefficient and p values were calculated using the cor.test function in R. An FDR-corrected $p < 0.05$ was considered significant. To determine the linear relationship between diversity index and clinical indices, such as IgA, linear regression analysis was performed using the lm and cor test functions in R, and P values <0.05 were considered significant.

2.9. Microbial dysbiosis analysis

To determine the degree of microbial dysbiosis, the microbial dysbiosis index (MD index) was computed per Gevers et al. (2014). Here, the MD index was determined as the log of [total abundance of organisms increased in schizophrenia patients]/[total abundance of organisms decreased in controls] for all samples.

2.10. Receiver operating characteristic analysis

To determine whether metagenomic compositions, such as the MD index, IgA and glutamate synthase (GOGAT) can be used as biomarkers for schizophrenia, scikit-learn, a Python-based machine learning method with an L1-regularized logistic regression model was subjected to regression fit analysis, and the receiver operating characteristic (ROC) with 6-fold cross validation was used, and the area under the curve (AUC) was computed.

2.11. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was performed as previously described (Zhou et al., 2018a). Briefly, stool IgA contents were measured using the Salimetrics® SIgA Indirect Enzyme Immunoassay Kit (Salimetrics, Carlsbad, CA, USA) per the manufacturer's instructions. Glutamate synthase (GOGAT) activity was measured using a glutamate synthase activity assay kit (Cat. No. BC0075, Solarbio Science & Technology Co., Ltd, Beijing, China) per the manufacturer's instructions.

3. Results

3.1. Gut microbiota dysbiosis in patients with schizophrenia

Eighty-four patients with schizophrenia and 84 healthy controls were enrolled in the study, and their demographic information and sample statistics are shown in Table 1, samples clinical information and sequencing statistics are detailed in Supplementary Table 1. The study was divided into two phases. In the discovery phase, shotgun metagenome sequencing was performed on 40 patients with schizophrenia and 40 healthy controls, and metagenome composition at the taxonomic and functional levels associated with schizophrenia were identified by a metagenome-wide association study. In the validation phase, 16S rRNA gene sequencing was performed on the remaining 44 patients with schizophrenia and 44 healthy controls to validate the metagenome composition. Fig. 1 details the bioinformatics analysis process. Considering that diet and lifestyle are important confounders of gut microbiota, we also investigated the differences in diet and lifestyle habits between SCZ and HC. The results are shown in Supplementary Table 2, and no significant differences were found.

The metagenomic information analysis obtained gut microbiota composition at the taxonomic and functional level (Supplementary Table 3). The gut microbiota diversities were compared between the patients with schizophrenia and the healthy controls, and the results showed that gut microbiota richness (Chao 1) was significantly decreased in patients with schizophrenia compared with healthy controls (Fig. 2a). NMDS analysis of the gut microbiota compositions was performed at the species level, and patients with schizophrenia were easily distinguished from the healthy controls, it should be noticed that not all schizophrenia patients could be distinguished in the NMDS analysis – there were a few samples that clustered with the normal control samples (Fig. 2b). To systematically understand the specific differences in gut microbiota compositions between the patients with schizophrenia and the healthy controls, metagenomic association analysis (MWAS) was firstly performed based on 40 SCZ and 40 HC stool samples, then, an additional 44 SCZ and 44 HC stool samples were collected for 16S sequencing (OTUs data was shown Supplementary Table 4), finally 19 significantly different taxonomies were found Supplementary Table 5), 12 of which were significantly increased in patients with schizophrenia compared with healthy controls: phylum of *Actinobacteria*, class of *Deltaproteobacteria*, orders of *Actinomycetales* and *Sphingomonadales*, family of *Sphingomonadaceae*, genus of *Eggerthella* and *Megasphaera*, species of *Akkermansia muciniphila*, *Bifidobacterium adolescentis, Clostridium perfringens*, *Lactobacillus gasseri* and *Megasphaera elsdeniis*. Seven taxonomies were significantly decreased in patients with schizophrenia compared with healthy controls: order of *Rhodocyclales*, families of *Alcaligenaceae*, *Enterococcaceae*, *Leuconostocaceae*, *Rhodocyclaceae* and *Rikenellaceae*, genus of *Enterococcus*. The MD indexes were computed based on these taxonomies, and the results showed that the MD index was significantly increased in patients with schizophrenia compared with that of healthy controls (Fig. 2c). The MD index was also significantly negatively correlated with Chao 1 (Fig. 2d).

MWAS was also performed to identify the differences in the gut microbiota compositions at the functional level between patients with schizophrenia and the healthy controls. Thirty-four significantly differentially abundant functional pathways were found (Supplementary Table 6), of which, 15 were significantly increased in patients with schizophrenia, and 19 were significantly decreased in patients with schizophrenia compared with healthy controls. As expected, most differential pathways were significantly associated with differential taxonomies. The impact of important clinical phenotypes, such as sex, on the gut microbiota compositions were evaluated, and the results showed that sex had a limited impact on MD index (Supplementary Fig. 1).

3.2. Altered gut microbiota-associated epitopes in patients with schizophrenia

We previously showed that gut MEs may represent gut microbiota-specific antigens in neuropsychiatric disorders, such as autism spectrum disorder (Wang et al., 2019); thus, we predicted the ME compositions in the shotgun metagenomic-sequenced samples, and the ME diversity indexes were compared between patients with schizophrenia and

Table 1
Demographic and Clinical Characteristics of the Study Subjects.

	Discovery		*p*	Validation		*p*
	SCZ	HC		SCZ	HC	
Number (n)	40	40	NA	44	44	NA
Sex (female/male)	20/20	20/20	1	16/28	16/21	0.64895
Age (in years)	35 ± 11	34 ± 9	0.81439	35 ± 11	35 ± 11	0.91219
BMI	NA	NA	NA	22 ± 3.21	23.09 ± 3.71	0.1455
Method	Metagenome	Metagenome	NA	16S rRNA	16S rRNA	NA
GOGAT (nmol/min/g)	NA	NA	NA	1300 ± 850	790 ± 600	0.00195
IgA (OD 450 nm)	NA	NA	NA	320 ± 170	110 ± 95	1.16E−08

SCZ, schizophrenia; HC, healthy controls; NA, not available; GOGAT, glutamate synthase.

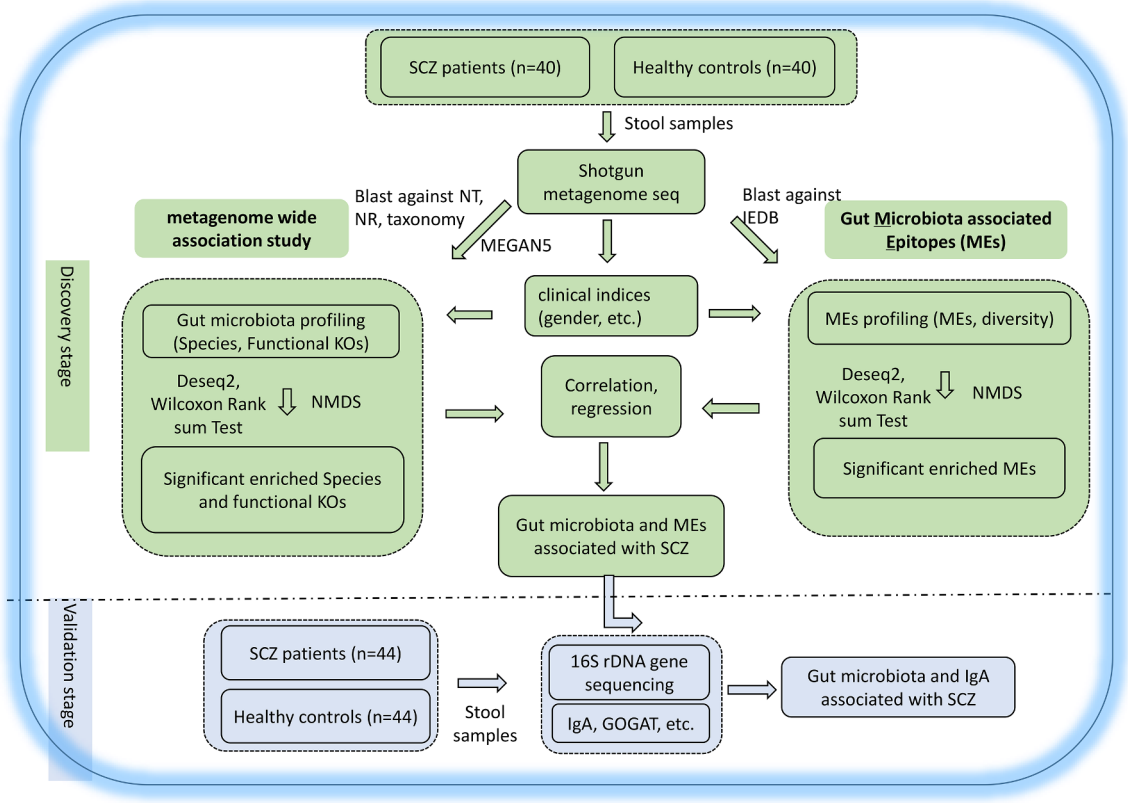

Fig. 1. Flowchart of the study design. Forty patients with SCZ and 40 healthy controls were enrolled. Fecal samples were collected from the participants, and shotgun metagenomic sequencing was performed. Clean reads without human sequences were blasted to NCBI's, NT, NR and taxonomy databases, and MEGAN5 analysis was used to obtain the gut microbiota compositions at the taxonomic and functional levels. Clean reads were blasted to the IEDB database to identify the MEs. Finally, 44 SCZ patients and 44 healthy controls were enrolled for validation. Regression and correlation analysis were used to assess the relationship between differential gut microbiota compositions/ME compositions (ME and diversity index) and/or IgA, and ROC curve analysis was performed to evaluate whether MD index, IgA, and GOGAT could be potential biomarkers for SCZ. Abbreviations: KO, KEGG Orthology; SCZ, schizophrenia; MEGAN5, metagenomic analysis software METAGenome Analyzer version 5.0; MWAS, metagenomic association study; NMDS, nonmetric multidimensional scale method; NCBI, National Center for Biotechnology Information; NT, nucleotide; NR, nonredundant protein; MEs, microbiota-associated epitopes; ROC, receiver operating characteristic; GOGAT, glutamate synthase; IEDB, Immune Epitope Database.

healthy controls. The results showed that the ME diversity was significantly higher in patients with schizophrenia than in the healthy controls (Fig. 3a, $P < 0.001$) and that ME diversity was also positively correlated with MD index (Fig. 3b, Supplementary Fig. 2). Because IgA is an indicator of gut immune-associated microbiota, gut IgA levels were determined (Zhou et al., 2018a) and compared between patients with schizophrenia and healthy controls. The results showed that gut IgA levels in patients with schizophrenia were higher than those of healthy controls (Fig. 3c, $P < 0.001$), and IgA levels were positively correlated with the MD index (Fig. 3d), indicating that the alteration in gut microbiota-associated mucosal immunity was associated with altered gut microbiota compositions.

To identify specific ME markers that distinguish patients with schizophrenia from healthy controls, MWAS was performed, and 30 differential MEs were found (Supplementary Table 7), most of which were closely related to the different gut microbiota compositions (Fig. 3e). Interestingly, ANYGYTFGSGTRLTVV, TVTSAHPEDSSFYICS, TVTSAQKNPIAFYLCA, and SARDLTSGANNEQFFGPGTRLTVL, all are similar to the amino acid sequence of the T-cell receptor beta chain, were positively correlated with MD index (Supplementary Fig. 2).

3.3. Altered gut glutamate metabolism in patients with schizophrenia

Considering the altered gut microbiota composition at the functional level in patients with schizophrenia, including the significant increase in abundance of proline dehydrogenase involved in glutamate metabolism (Supplementary Fig. 3a), GOGAT activity was determined in the feces. The results showed that GOGAT activity was significantly higher in the guts of patients with schizophrenia than in those of the healthy controls (Supplementary Fig. 3b). GOGAT activity and *Deltaproteobacteria* were positively correlated (Supplementary Fig. 3c), while GOGAT activity and *Rhodocyclales* were negatively correlated (Supplementary Fig. 3e). This increase in *Deltaproteobacteria* and decline in *Rhodocyclales* was associated with increased IgA levels (Supplementary Fig. 3d and f). Altered gut glutamate metabolism in patients with schizophrenia may be associated with gut immune alterations.

3.4. Gut markers in patients with schizophrenia

Finally, machine learning was used to evaluate the performances of the MD index, IgA and GOGAT used as gut markers of schizophrenia, and the results showed that the AUC reached 0.86 (Fig. 4), the distinguishing effect of MD Index, IgA and GOGAT may have similar effects to the significant differential taxonomies (Supplementary Figs. 4 and 5). Indicating that they are potential gut markers of schizophrenia. Our findings also imply that regulating the gut microbiota composition and immunity may be novel approaches to treating schizophrenia.

4. Discussion

Evidence has shown that immune imbalance may play a role in the

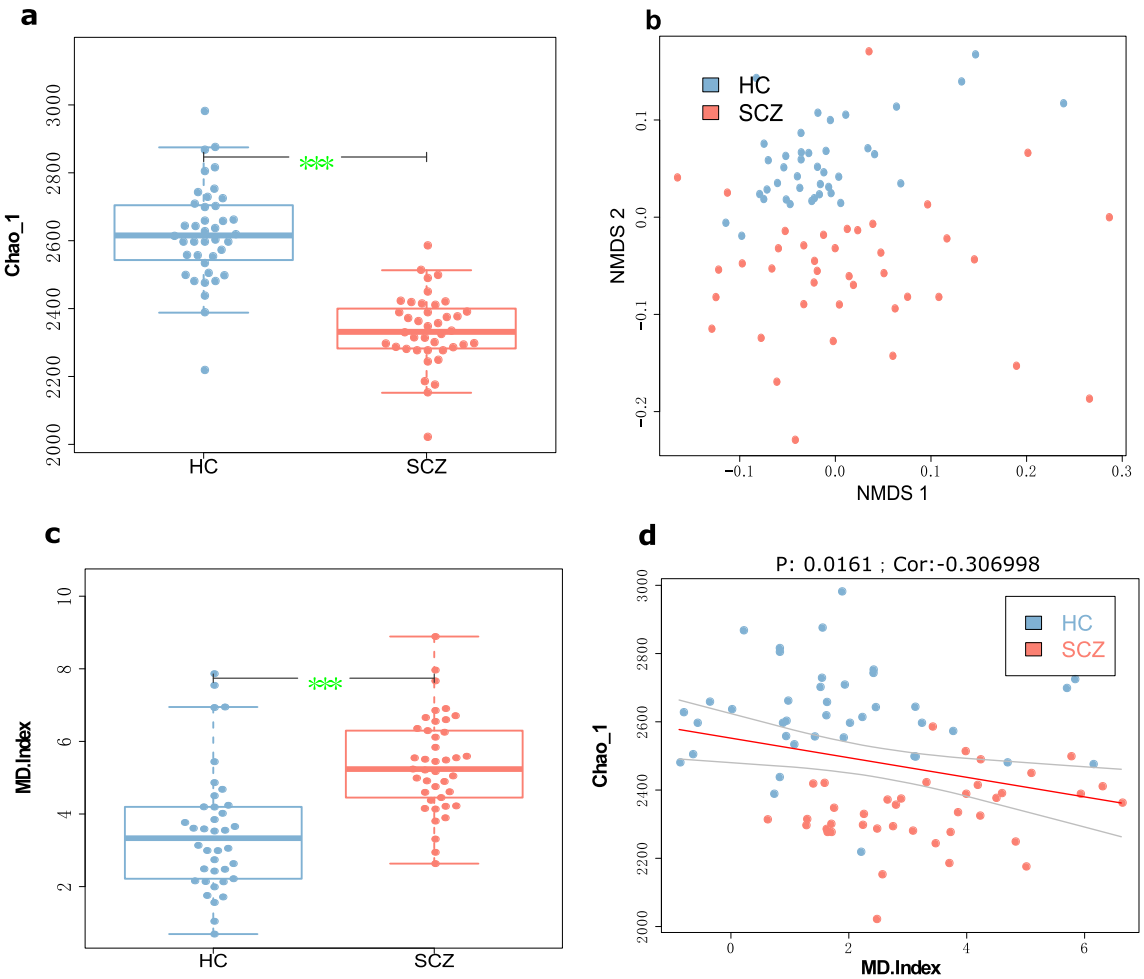

Fig. 2. Altered gut microbiota composition in patients with schizophrenia. All data comes from the discovery phase, a) gut microbiota richness (Chao 1) is significantly lower in the guts of patients with schizophrenia (SCZ) than those of healthy controls (HC), "*", $p < 0.05$ by the Wilcoxon rank-sum test; b) Nonmetric multidimensional scale method (NMDS) analysis showed that the gut microbiota composition (species level) can distinguish SCZ samples from HC samples; c) gut microbial dysbiosis index (MD index) in patients with schizophrenia is significantly higher than that of healthy controls, "***", $p < 0.001$ by the Wilcoxon rank-sum test; d) the decrease in gut microbiota richness (Chao 1) in patients with schizophrenia is associated with an increased MD index.

pathogenesis of schizophrenia (Horvath and Mirnics, 2014; Severance et al., 2012), and new evidence suggests that the gut microbiota may play an important role in the pathogenesis of various complex diseases, including schizophrenia, through the "gut-brain" axis (Dickerson et al., 2017); however, the role of the gut microbiota in schizophrenia with a suspected immune imbalance is unclear. In the present study, we found that gut microbiota compositions in patients with schizophrenia were characterized by significant increases in ME diversity and associated with significantly increased gut IgA levels. This may imply that innate immune imbalances in schizophrenia and the gut microbiota have important roles in the immune imbalances in schizophrenia. Our results support previous studies by Severance *et al.*, who found that the serum markers of bacterial translocation, including soluble CD14 and lipopolysaccharide-binding protein, were significantly elevated in patients with schizophrenia and were significantly associated with elevated C-reactive protein in the serum (Severance et al., 2013). Furthermore, regulating the gut microbiota may be a potential therapy for schizophrenia. Severance *et al.* found that serum antibodies against *Candida albicans* were elevated in patients with schizophrenia, and probiotic treatment can significantly reduce these antibody levels and effectively relieve the patient's psychiatric symptoms (Severance et al., 2017).

Several reports on the gut microbiota composition in patients with schizophrenia used the 16S rRNA gene sequencing method and lacked independent validation samples (Nguyen et al., 2019; Shen et al., 2018). We, for the first time, used shotgun metagenomic sequencing to identify gut microbiota composition associated with schizophrenia and validate them in additional samples. Nineteen taxonomic markers were associated with schizophrenic patients, and the MD index based on the abundance of these taxonomic markers can be used to reflect the degree of gut microbiota dysbiosis in patients with schizophrenia. The MD index was also associated with altered gut immunity in patients with schizophrenia. We found that the abundances of *Megasphaera*, *Megasphaera elsdenii* and *Clostridium perfringens* in the guts of patients with schizophrenia were significantly higher than those of healthy controls, which is consistent with the findings of Shen et al. (2018). *Megasphaera* has been linked to poor cognition and inflammation in patients with hepatic encephalopathy (Bajaj et al., 2012), and *Clostridium perfringens* is a ubiquitous pathogen that produces many toxins (Petit et al., 1999). We also found that *Lactobacillus gasseri* and *Bifidobacterium adolescentis* were significantly enriched in patients with schizophrenia, which is consistent with the findings of Castro-Nallar et al. (2015). Studies have shown that lactic acid bacteria, such as *Lactobacillus* and *Bifidobacterium*, can regulate chronic stress-induced inflammation and behavioral changes (Braat et al., 2004; Moya-Perez et al., 2017); increased abundances of these bacteria in the gut may imply gut inflammation. It is worth noting that we found that four SCZ-enriched MEs similar to human TCR sequences are positively correlated with SCZ-enriched bacteria *Clostridium perfringens* and *Lactobacillus gasseri*. Our results may

also support the hypothesis that SCZ-enriched gut bacteria may be associated with SCZ-associated inflammatory responses.

Studies have shown that schizophrenia is associated with elevated glutamatergic metabolites in the brain (Merritt et al., 2016), but the specific mechanism is unknown. We found that the activity of gut glutamate synthetase (GOGAT) was significantly elevated in patients with schizophrenia, which is consistent with the results of Castro-Nallar et al. (2015), who found a significant increase in the abundance of oropharynx microbiota functional pathways associated with glutamate metabolites in patients with schizophrenia. GOGAT catalyzes the amino transfer of glutamine to α-ketoglutaric acid to form two glutamic acid molecules, and the increased GOGAT activity can directly lead to an

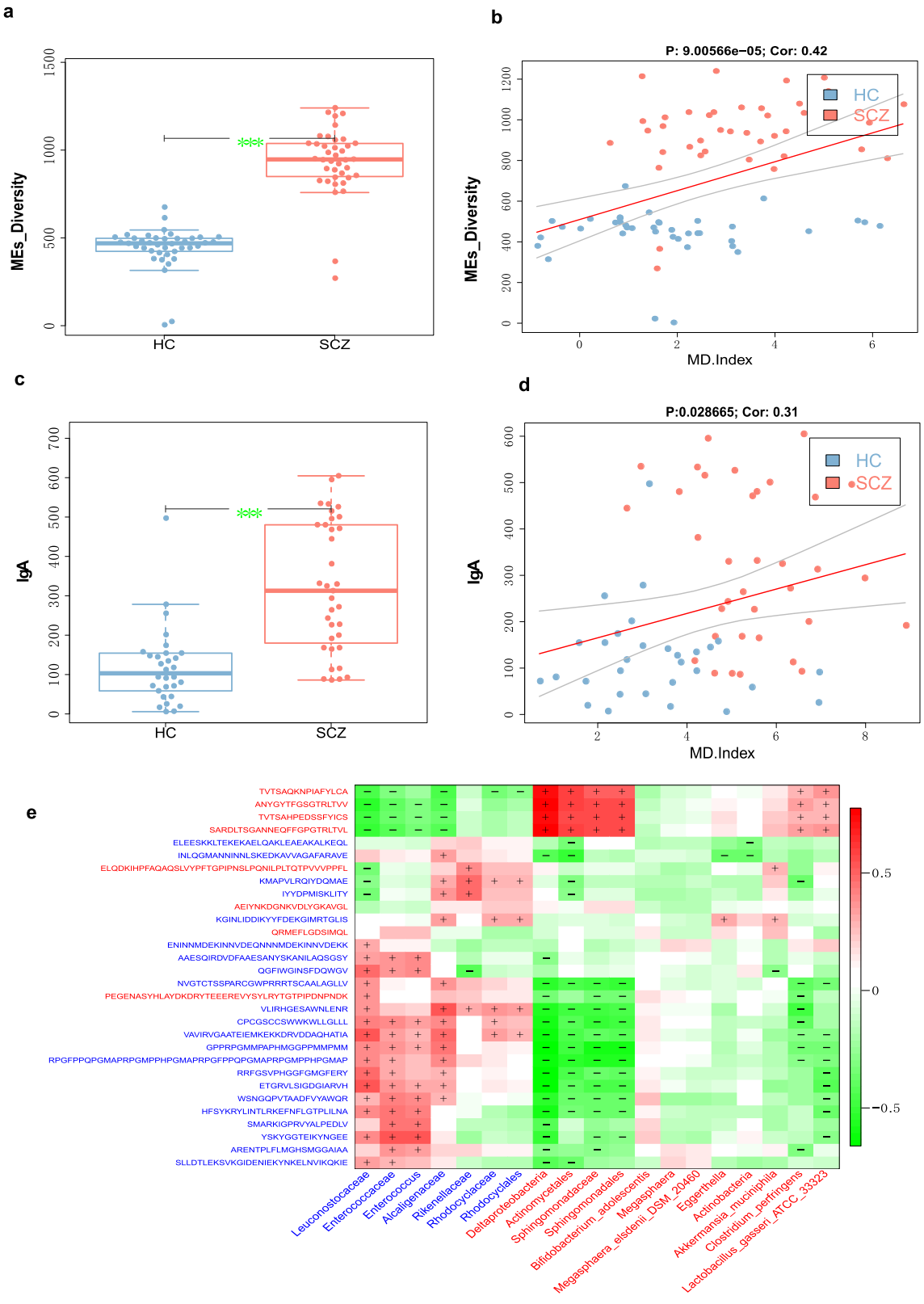

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Fig. 3. Altered gut microbiota composition associated with gut microbiota-associated epitopes in patients with schizophrenia. a) gut microbiota-associated epitope (ME) diversity in guts of patients with schizophrenia is significantly higher than that of healthy controls (HC), the data comes from the discovery phase, "***", $p < 0.001$ by the Wilcoxon rank-sum test; b) Elevated ME diversity in patients with schizophrenia is associated with an increased microbial dysbiosis (MD) index, the data comes from the discovery phase; c) IgA levels in guts of patients with schizophrenia is significantly higher than that of healthy controls, the data comes from the validation phase, "***", $p < 0.001$ by the Wilcoxon rank-sum test; d) IgA levels are positively correlated with MD index, the data comes from the validation phase; e) differential MEs are correlated with differential gut microbiota taxonomies, SCZ increased and decreased taxonomies/MEs are marked as red and blue, respectively, the data comes from the discovery phase, right panel is the scale of the spearman correlation coefficient, red and blue represent positive and negative correlations, respectively; "+" and "−" represent significant positive and negative correlations, respectively; ME abundance of TVTSAQKNPIAFYLCA, ANYGYTFGSGTRLTVV, TVTSAHPEDSSFYICS, SARDLTSGANNEQFFGP GTRLTVL were significantly positively associated with the taxonomies: *Deltaproteobacteria*, *Actinomycetales*, *Sphingomonadaceae*, and *Sphingomonadales*, which were elevated in guts of patients with schizophrenia. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

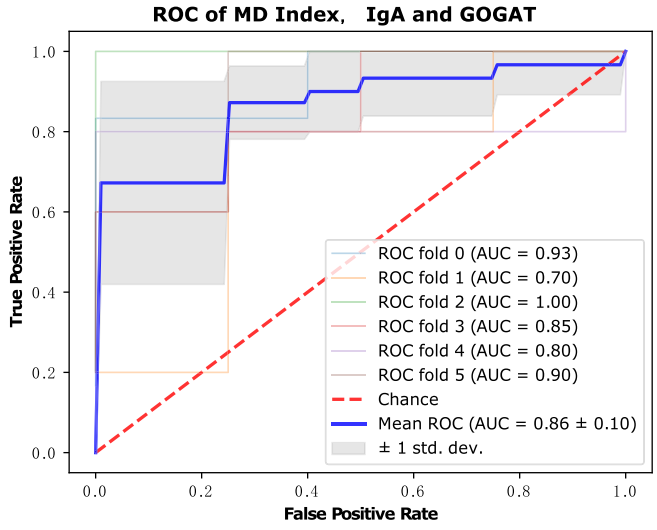

Fig. 4. MD Index, IgA and GOGAT were potential gut markers in patients with schizophrenia. All data comes from the validation phase. Receiver operating characteristic (ROC) curves with 6-fold cross validation for the L1 penalty logistic regression model. The blue line is the mean ROC curve, the gray interval is the confidence interval of the standard deviation, and the average area under the ROC curves (AUC) is 0.86, indicating good performance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

increase in glutamate. At the same time, our results are consistent with the results of Zheng et al. (2019), which found altered glutamate metabolism in patients with schizophrenia and confirmed a functional link between microbiome and behavior in gnotobiotic mice, We also found that the increased GOGAT activity was associated with an increased abundance of *Deltaproteobacteria*, and this increase was associated with increased IgA, suggesting that changes in intestinal glutamate metabolism are closely related to changes in the gut microbiota immunity. At present, progress has been made in the research on the microbiome of schizophrenia (summarized in Supplementary Table 8), these studies together with the present study suggest that the microbiome, especially the gut microbiome, may play an important role in the etiology of schizophrenia.

Some innovations in the present study include using the shotgun method for the first time to study gut microbiota compositions in patients with schizophrenia and validate them in additional samples. We used a shotgun metagenomic dataset to predict the potential gut microbiota-associated epitopes (MEs) in patients with schizophrenia. This study also had some limitations. The MEs we predicted remain to be validated, and in-depth functional studies are needed. In summary, our findings suggest altered gut microbiota and mucosal immunity in patients with schizophrenia, and intestinal markers, such as the MD index, IgA, and GOGAT, may be potential biomarkers for patients with schizophrenia. Although it is difficult to provide a view of the causal relationship between gut microbiota and schizophrenia, our results may inform our understanding of a role for the microbiome in schizophrenia etiology and contribute towards microbiome targeted interventions for schizophrenia.

Data availability

The data that support the findings of this study have been deposited in the CNGBdb (https://db.cngb.org) with accession number CNP0000401.

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All authors read and approved the final manuscript, and all authors report no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2019.06.039.

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